The authors are grateful to E. B. Towne and L. D. Apperson for assistance.

CHEMICAL LABORATORY
IOWA STATE COLLEGE
AMES, IOWA
RECEIVED MARCH 27, 1939

The Reduction of α-Bromocyclohexanone with Aluminum Isopropoxide

BY S. WINSTEIN

The reduction of some α-bromo ketones by aluminum isopropoxide was the subject of a recent communication to the Editor by Stevens,[1] who obtained from α-bromopropiophenone a 35% yield of bromohydrin and about an equal yield of product not containing bromine. Presumably hydrogen bromide was split out. Tertiary α-bromo ketones and cyclic secondary α-bromo ketones were reported to yield not bromohydrins but products almost entirely free of bromine. What type of product he obtained was not indicated. In the course of other work the author has had occasion to reduce with aluminum isopropoxide the cyclic secondary α-bromo ketone, α-bromocyclohexanone. Since Stevens is continuing his investigation of the reaction of α-bromo ketones with aluminum isopropoxide, the results obtained with this cyclic ketone should be reported.

The reaction product from the reduction of α-bromocyclohexanone was found to be a mixture of bromohydrin and cyclohexanol, in yields of 30 and 33%, respectively, with no unsaturated compound being isolated. It is possible that the cyclohexanol arises from dismutation of bromocyclohexanone to cyclohexanone and dibromocyclohexanone with subsequent reduction of the cyclohexanone to cyclohexanol.

(1) Stevens, THIS JOURNAL, **60**, 3089 (1938).

Experimental

75.8 g. (0.428 mole) of α-bromocyclohexanone, b. p. 69–71° (1.5 mm.), prepared by the method of Kötz,[2] dissolved in 200 ml. of anhydrous isopropanol (Shell) was added to aluminum isopropoxide solution prepared from 7.5 g. of aluminum and 75 ml. of anhydrous isopropanol, according to the directions of Young, Hartung and Crossley.[3] The mixture was refluxed for three and one-half hours. Then it was concentrated to a thick residue by distillation first of acetone, then of solvent through a 20-cm. column of glass helices for two hours at atmospheric pressure and finally with the aid of an aspirator. One hundred ml. of water and 130 ml. of 6 *N* sulfuric acid were added to the residue and all lumps were broken up. A little ether was added and the oil phase was separated, washed with bicarbonate solution and dried over sodium sulfate. Distillation and then refractionation at reduced pressure through a 40-cm. Weston[4] column yielded 22.6 g. (30%) of 2-bromocyclohexanol, b. p. (10 mm.) 85.5–86.5°, n^{25}D 1.5164, and 14.3 g. (33%) of cyclohexanol, b. p. (10 mm.) 61.0–61.2°, n^{25}D 1.4649, m. p. of 3,5-dinitrobenzoate and mixed m. p. with authentic specimen, 112°.

GATES AND CRELLIN LABORATORIES OF CHEMISTRY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIF.
RECEIVED MARCH 21, 1939

(2) Kötz, *Ann.*, **358**, 195 (1907).
(3) Young, Hartung and Crossley, THIS JOURNAL, **58**, 100 (1936).
(4) Weston, *Ind. Eng. Chem., Anal. Ed.*, **5**, 179 (1933).

COMMUNICATIONS TO THE EDITOR

COLOR REACTIONS IN VITAMIN K CONCENTRATES

Sir:

During studies of the inactivation of vitamin K by its reaction with bases, we have detected and separated an alcohol-soluble reddish pigment. Recently, Dam, *et al.* [*Helv. Chim. Acta*, **22**, 310 (1939)] described a color reaction of vitamin K concentrates with sodium ethylate in which a transient purple color changing to a reddish-brown color developed. We have determined that our pigment is the end stage of this color reaction and that the quantity of pigment formed is closely correlated with antihemorrhagic activity. The transient, deep purple color is considerably masked when carotenoid pigments are present; however, it is possible to employ the final, less intense but relatively stable, reddish-brown color as a quantitative measure of the vitamin.

The color reaction is carried out easily by dissolving a few milligrams of concentrate in 1 or 2 cc. of methanol and then adding 1 cc. of sodium methylate (2 to 3 g. of sodium dissolved in 50 cc. of methanol). When warmed for a few minutes, the mixture slowly develops a distinct purple

color, if sufficient vitamin K is present and interfering pigments are practically absent. Soon the color changes to a reddish-purple and finally to a reddish-brown. At this point, carotenoid pigments may be removed by partition with a hydrocarbon solvent. The color due to reaction of the vitamin with sodium methylate remains in the methanol phase. To test the agreement of color reaction with activity, we have applied this reaction to a variety of sources of the vitamin assayed by a procedure already described [*Biochem. J.*, **32,** 1897 (1938)]. Results are given in Table I.

Fractions obtained by chromatographic adsorption showed a consistent relation of color test to activity. This was also true of fractions obtained by incomplete molecular distillation and of a preparation (concentrate 1270) obtained by repeated precipitation from methanol by chilling with solid carbon dioxide [*J. Biol. Chem.*, **120,** 635 (1937)] but not purified from sterols. A preparation of the molecular compound of the vitamin with deoxycholic acid [THIS JOURNAL, **61,** 745 (1939)] showed a color reaction consistent with its activity, which was also true of the residue of this preparation remaining after partial extraction of the vitamin with xylene. A strong color reaction was also produced on testing an active concentrate prepared by repeated molecular distillation of soybean oil, followed by removal of sterols, free fatty acids and waxes. In addition to the data in the table, we may report that the color reaction has been obtained in extracts of several kinds of bacteria known to be good sources of the vitamin [*Proc. Soc. Exp. Biol. Med.*, **38,** 336 (1938)].

TABLE I

ANTIHEMORRHAGIC ACTIVITY AND COLOR REACTION INTENSITY OF VITAMIN K CONCENTRATES

	Concentrate	Level fed per kilo of diet, mg.	Av. blood clotting time, min.	Rel. intensity of color test
	Chromatographic adsorption fractions			
1	Orange zone	10	2.8	4
2	Light yellow zone	10	1.8	8
3	Yellow zone	10	6.3	2
4	Colorless zone	10	>30	0
	Incomplete distillation fractions			
1	Low temp. distillate, P11	80	>30	0
2	Vitamin distillate, P11	20	4.2	2
3	Residue, P11	20	7.3	1
4	Vitamin distillate, P8	10	3.5	4
5	Residue, P8	10	3.7	4
6	Vitamin distillate, P12	20	3.7	2
	Other preparations			
1	Concentrate 1270	10	4.7	4
2	K-choleic acid, 8P	30	2.7	4
3	K-choleic acid, 8P xylene extracted	30	14.1	1
4	Soybean oil concentrate	400	3.0	strong

The results strongly indicate that the color reaction is due to the vitamin itself. The character of the pigment is being studied further.

DIVISION OF POULTRY HUSBANDRY
DEPARTMENT OF AGRICULTURE
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BERKELEY, CALIF.
H. J. ALMQUIST
A. A. KLOSE

RECEIVED MAY 19, 1939

THE ANTI-HEMORRHAGIC ACTIVITY OF PURE SYNTHETIC PHTHIOCOL

Sir:

We wish to announce the discovery of the anti-hemorrhagic activity of pure synthetic phthiocol, 2-methyl-3-hydroxy-1,4-naphthoquinone. The physical and chemical properties of this compound are in general similar to those known for vitamin K. When fed to chicks at a level of 20 mg. per kg. of vitamin K-free diet, phthiocol maintained the average blood-clotting time at 2.1 minutes in one test and 1.6 minutes in a second test. At a level of 10 mg. the blood-clotting time was maintained at 1.8 minutes. Chicks fed only the basal ration had prolonged blood-clotting times. The minimum required level is being determined. It is probable that phthiocol is the simplest member of an homologous series of anti-hemorrhagic substances.

We are indebted to Professor R. J. Anderson for the phthiocol used in these experiments.

DIVISION OF POULTRY HUSBANDRY
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BERKELEY, CALIF.
H. J. ALMQUIST
A. A. KLOSE

RECEIVED MAY 31, 1939

CHROMATOGRAPHIC ADSORPTION AND DIPOLES

Sir:

The use of the method of chromatographic adsorption has become extremely important for the separation of complex mixtures of organic molecules.

A careful survey of numerous experimental investigations has revealed the importance of dipoles in determining the order of adsorption of a mixture on a polar medium (*i. e.*, aluminum oxide). Thus Karrer [Karrer and Nielsen, *Ber.*

Ges. Physiol. Exptl. Pharmakol., **86**, 529–530 (1935)] has shown that on aluminum oxide the order of occurrence in the tube of mono-nitrophenols is para, meta, and ortho. These authors obtained similar results with the corresponding nitroanilines. This proves that basicity and acidity are not important factors. The above order, however, is exactly that of the decreasing permanent dipoles of these substances. Cook [*J. Chem. Soc.*, 876 (1938)] has shown recently that *cis*-azobenzene is more strongly adsorbed on alumina than the *trans* modification. This is in accord with this author's idea concerning the dipole interaction between the solute dipoles and the fixed dipoles in the polar adsorbing media.

In cases where no permanent dipole exists, it is to be expected that those substances with highest polarizability (*i. e.*, ease to form an induced dipole) would be more strongly adsorbed. This is actually the case with Kuhn's polyenes.

Adsorption of this type is competitive between the solvent and adsorbing media.

The number of isolated dipoles in a molecule is important, however. It has been shown here that picric acid with three nitro groups is more strongly adsorbed on aluminum oxide (from benzene–petroleum ether solutions) than is *o*-nitrophenol, although the latter has a larger permanent dipole. The same was found for 4-methyl-2-nitrophenol.

It appears that in isomeric molecules containing the same number and kind of functional groups, those with the larger dipole moments are more strongly adsorbed on polar media.

A detailed study is being made in this Laboratory and will be reported later.

DEPARTMENT OF CHEMISTRY RICHARD T. ARNOLD
UNIVERSITY OF MINNESOTA
MINNEAPOLIS, MINNESOTA
RECEIVED MAY 5, 1939

DERIVATIVES OF VITAMINS K_1 AND K_2

Sir:

As further evidence that we have actually isolated vitamins K_1 and K_2 and that the two vitamins contain the suggested quinone structure [THIS JOURNAL, **61,** 1295 (1939)], we have now prepared the diacetates of dihydro vitamin K_1 and dihydro vitamin K_2. These diacetates are colorless, crystalline derivatives which possess the activity of vitamin K. When vitamin K_1 is reductively acetylated the diacetate of dihydro vitamin K_1 is obtained. This derivative may be crystallized readily from low boiling petroleum ether (30–60°) or methyl alcohol (solvents in which vitamin K_1 is soluble and insoluble, respectively) in fine white needles melting at 59°. *Anal.* Found: C, 78.21, 78.01; H, 10.07, 10.03; mol. wt., 531. Calcd. for $C_{36}H_{56}O_4$: C, 78.21; H, 10.21; mol. wt., 552; for $C_{36}H_{54}O_4$: C, 78.50; H, 9.88; mol. wt., 550. Microhydrogenation: uptake of H_2, 3.04 moles (of vitamin K_1 4.08 moles). Assay: approximately 500 units per mg. There is general absorption in the region from 220 mμ to beyond 300 mμ with intense absorption at 230 mμ where the extinction coefficient of $E^{1\%}_{1\,cm.}$ = 1250.

The compound is not readily hydrolyzed by alkali or acid in an aqueous or alcoholic medium. In alcoholic solution its activity is unstable to 1% potassium hydroxide and thirty-six hours of exposure to sunlight but is stable to one hundred hours of exposure to light from a 100-watt bulb at a distance of 4 feet (1.2 meters).

Diacetyl dihydro vitamin K_1 was converted to vitamin K_1 by treating it with an excess of methylmagnesium iodide followed by shaking an ether solution of the hydrolyzed product with air. After fractionation by distillation at 2×10^{-4} mm. pressure, 85–90% of the theoretical yield of the vitamin was obtained. *Anal.* Found: C, 82.34; H, 10.13. Calcd. for $C_{32}H_{48}O_2$: C, 82.70; H, 10.41; for $C_{32}H_{50}O_2$: C, 82.33; H, 10.80. Assay: 1000 units per mg.

Repetition of the reductive acetylation of this vitamin K_1 preparation gave a compound which according to melting point, mixed melting point and bio-assay was identical with the original diacetate of vitamin K_1.

Vitamin K_2 was converted to the diacetate of dihydro vitamin K_2 by the same method as used for K_1. The derivative after purification by several recrystallizations from low boiling petroleum ether (30–60°) and methyl alcohol melted at 57–58°. *Anal.* Found: C, 80.89, 81.03; H, 9.94, 9.79; mol. wt., 628. Calcd. for $C_{44}H_{60}O_4$: C, 80.93; H, 9.26; mol. wt., 652; for $C_{44}H_{62}O_4$: C, 80.68; H, 9.54; mol. wt., 654. Microhydrogenation: uptake of H_2, 7.99 moles; (of vitamin K_2 8.73 moles). Assay: approximately 300 units per mg. The ultraviolet absorption is very similar to that of the diacetate of dihydro vitamin K_1. The extinction coefficient of $E^{1\%}_{1\,cm.}$ = 1250 at 232 mμ.

In our previous communication (*loc. cit.*) we gave an extinction coefficient of $E^{1\%}_{1\,cm.}$ = 385 at 248 mμ for vitamin K_1 but since that time a value of 540 has been obtained. The analyses of both preparations indicated that each was analytically pure. Our first value was probably due to instability of the vitamin on storage and toward light. For this reason we are not at present certain that 540 is the maximum value attainable. In this same communication line 38 column 1 should read "all have a potency of about *1000* units per mg." instead of, "all have potency of about 100 units per mg."

BIOCHEMISTRY DEPARTMENT
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S. B. BINKLEY
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R. W. MCKEE
E. A. DOISY

RECEIVED MAY 22, 1939

DENATURATION OF MYOSIN

Sir:

Myosin is a protein particularly susceptible to "denaturation" by very mild chemical agents. We have employed four criteria of alteration in the molecule: alteration in –SH groups,[1] loss of double refraction of flow,[2] changes in viscosity and in solubility. Seven preparations of myosin from rabbit muscle, and one from lobster, have been studied with very consistent results.

Some of our observations are briefly summarized in Table I, from which several conclusions may be drawn. (1) The extreme asymmetry of the molecule, on which its double refraction depends, is diminished rapidly by all commonly employed denaturing agents, and by many other substances as well. (2) The content of titratable –SH groups is greatly increased in concentrated solutions of urea and even more in guanidine hydrochloride; in the presence of the latter at 16 *m*, nearly all the non-methionine sulfur of myosin[3] can be accounted for as –SH. The concentrations of urea and guanidine needed to produce marked increase in –SH content are much greater than those needed to destroy double refraction. (3) Other substances, such as lithium, calcium and magnesium chlorides, destroy double refraction but do not affect –SH groups. Substances containing an ammonium group abolish titratable –SH completely (but the full content of titratable –SH is immediately restored by adding concentrated guanidine hydrochloride). Such substances may or may not destroy double refraction (see table). There appears to be no systematic correlation between the effect of reagents on –SH groups and their effect on double refraction.

(1) Greenstein, *J. Biol. Chem.*, **125**, 501 (1938); **128**, 233 (1939).
(2) Von Muralt and Edsall, *ibid.*, **89**, 351 (1930).
(3) Bailey, *Biochem. J.*, **31**, 1406 (1937).

TABLE I

EFFECT OF REAGENTS ON DOUBLE REFRACTION OF FLOW AND ON SULFHYDRYL CONTENT OF RABBIT MYOSIN

All substances tested were added to myosin dissolved in KCl, 0.4–0.5 *n*, at *p*H 6.2–7.4. *T* denotes the time required for disappearance of double refraction of flow. –SH content is expressed as percentage cysteine.

Substance added	Molality in solution	*T*	–SH content, %
KCl	0.5	>2 weeks	0.42 ± 0.03
KBr	0.80	15 min.	..
KI	0.27	15 min.	0.46
Guanidine HCl	0.30	5 min.	0.42
Guanidine HCl	16.6	At once	1.14 ± 0.03
Guanidine HI	0.075	>5 days	..
Guanidine HI	0.14	1 hour	..
Guanidine HI	0.20	5 min.	..
Guanidine HI	0.28	<30 sec.	..
Urea	1.4	15 min.	0.42
Urea	16.6	At once	0.66 ± 0.03
LiCl	1.0	10 min.	0.46
$MgCl_2$	0.35	2 min.	0.46
$CaCl_2$	0.25	5–10 min.	..
NH_4Cl	1.4	10 min.	0
CH_3NH_3Cl	1.4	10 min.	0
Arginine mono-HCl	0.35	10 min.	0
Glycine	1.7	>1 week	0

The viscosity of myosin solutions decreases markedly on addition of reagents which destroy double refraction of flow. This is explicable on the assumption that the very long molecules of native myosin are broken up into smaller and less asymmetrical chains by the action of such reagents. This decrease in viscosity is in marked contrast to the increase produced by denaturation in solutions of "globular" proteins.[4]

The solubility of myosin is not fundamentally altered by any of the reagents studied; it retains the characteristics of a typical globulin. This is in marked contrast with the loss of solubility produced by heating myosin[5] and with the effect of all denaturing agents in decreasing the solubility of such proteins as egg albumin or hemoglobin.

(4) Anson and Mirsky, *J. Gen. Physiol.*, **15**, 341 (1932).
(5) Mirsky, *Cold Spring Harbor Symp. Quant. Biol.*, **6**, 150 (1938).

DEPARTMENT OF PHYSICAL CHEMISTRY
HARVARD MEDICAL SCHOOL
BOSTON, MASS.

JOHN T. EDSALL
JESSE P. GREENSTEIN
JOHN W. MEHL

RECEIVED MAY 17, 1939

ON THE COLOR REACTION FOR VITAMIN K

Sir:

Recently Dam, Karrer and co-workers [*Helv. Chim. Acta*, **22**, 310 (1939)] described a vita-

min K in a pure or nearly pure form. The vitamin was said to give a characteristic color reaction with sodium ethylate in alcoholic solution. We hoped that this reaction would be useful in the isolation of vitamin K from alfalfa, especially because the typical color changes described by the European workers were noticed with relatively crude concentrates. However, it was found that the color reaction is not a criterion for the presence of the vitamin as illustrated by the following experiment.

One gram of a vitamin K concentrate which gave the color reaction very strongly and had a potency of 1 unit [*J. Nutrition*, **17**, 303 (1939)] in 15 γ was dissolved in petroleum ether and chromatographically adsorbed on a slightly heat-activated calcium sulfate. Washing with petroleum ether was continued until the lowest yellow zone had passed into the filtrate. The adsorbed substance was then eluted with ether and we obtained 0.3 g. of a material with a very intense color reaction but with no vitamin K activity at 15 γ. The yellow filtrate contained 0.6 g. of an oil which did not give the typical color reaction; only a slight darkening occurred with the ethylate. However, it was fully active in a dose of 8 γ, containing the entire potency of the initial preparation.

Upon further purification by several chromatographic adsorptions on a more highly activated calcium sulfate, a concentrate was obtained which behaved like a single substance chromatographically. Its potency was comparable to that of the vitamin K_1 of McKee, *et al.* [THIS JOURNAL, **61**, 1295 (1939)], assuming that the potency of their product was not 100 but 1000 units per mg., as stated in their earlier paper [*Proc. Soc. Exptl. Biol. Med.*, **40**, 482 (1939)]. It was a light yellow oil which darkened on standing even in the refrigerator and which gradually lost potency. It did not give derivatives with reagents for keto groups or for hydroxyl groups, and in spite of its high degree of unsaturation failed to react with maleic anhydride in boiling benzene. Exposure to bright sunlight caused an almost instantaneous formation of a pink coloration fading in a few minutes. During the last steps of the isolation process the sodium ethylate color reaction became again positive, although it never reached the intensity of the previously separated inactive fraction. It may be that the blue coloration is given by readily formed decomposition products of vitamin K.

THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH
DIVISION OF ORGANIC CHEMISTRY
NEW BRUNSWICK, NEW JERSEY
ERHARD FERNHOLZ
S. ANSBACHER
MILDRED L. MOORE

RECEIVED MAY 19, 1939

THE RELATION BETWEEN METHYL *etio*-DESOXYCHOLATE AND THE METHYL DIHYDROXY-*etio*-CHOLANATE DERIVED FROM DIGOXIGENIN; METHYL 12-*epi*-*etio*-DESOXYCHOLATE

Sir:

Steiger and Reichstein [*Helv. Chim. Acta*, **21**, 828 (1938)] have degraded digoxigenin to a dihydroxy- and a diketo-*etio*-cholanic acid and a diketo-*etio*-cholenic acid. We have recently shown [THIS JOURNAL, **60**, 2824 (1938)] that these diketo acids are identical with the corresponding acids derived from desoxycholic acid. The dihydroxy acid, however, was found to be different from *etio*-desoxycholic acid in that its methyl ester melted at 180–183° while methyl *etio*-desoxycholate melted at 145–146°. Since both acids have the α configuration at C-3, epimerism at C-12 appeared to be the only plausible explanation of the difference. However, it became important to test this point in order to eliminate the possibility of a flaw in the other comparisons. The evidence now at hand confirms this explanation and furnishes additional proof for the positions of the oxygen atoms in the acids derived from digoxigenin.

Reduction of methyl 3,12-diketo-*etio*-cholanate in alcohol with platinum oxide catalyst resulted in a mixture of esters. The esters with the β configuration at C-3 were removed by precipitation with digitonin. The esters with the α configuration were separated by adsorption analysis on a column of alumina. Repeated crystallization of the fraction with higher melting point gave an ester (methyl 12-*epi*-*etio*-desoxycholate) which melted at 176–178°; $[\alpha]^{25}_{5461}$ + 49.4 ± 2.4° (0.203% in alcohol). Analysis of the ester was not entirely satisfactory because of the presence of ash (Calcd. for $C_{21}H_{34}O_4$: C, 71.96; H, 9.59. Found [corrected for ash]: C, 71.69; H, 9.81), but the 3-monobenzoate of the ester gave satisfactory values (Calcd. for $C_{28}H_{38}O_5$: C, 73.96; H, 8.42. Found: C, 74.06; H, 8.62). The monobenzoate melts at 136–138°; $[\alpha]^{25}_{5461}$ + 62 ± 3°.

Professor Reichstein very kindly compared

our ester with the one prepared by him from digoxigenin. He reported [private communication] that the melting points were identical and that the melting point of a mixture was not depressed. He has also supplied the specific rotation of his ester, $[\alpha]^{24}_{5461} + 45.6 \pm 3°$; $[\alpha]^{24}_D + 38.9 \pm 3°$ (1.183% in methanol).

The esters of three acids derived from digoxigenin have now been compared with the corresponding esters of known structure. The results show that digoxigenin has a hydroxyl group at C-12, the steric arrangement of which is opposite to that of the corresponding hydroxyl group of desoxycholic acid. A similar steric arrangement of the hydroxyl group at C-12 is present in the α-lagodesoxycholic acid described by Kishi [*Z. physiol. Chem.*, **238**, 210 (1936)].

THE MAYO FOUNDATION H. L. MASON
ROCHESTER, MINNESOTA W. M. HOEHN

RECEIVED MAY 18, 1939

THE ACTION OF PERIODIC ACID ON α-AMINO ALCOHOLS

Sir:

Periodic acid readily splits [Malaprade, *Bull. soc. chim.*, (5) **1**, 833 (1934)] substances carrying the grouping $-\underset{OH}{\overset{R}{C}}-\underset{OH}{\overset{R'}{C}}-$ (in which R or R′ may be H) to the ketones or aldehydes —C(=O)R and —C(=O)R′. This reaction recently has been applied very effectively to glucoside derivatives [Jackson and Hudson, THIS JOURNAL, **59**, 994, 2049 (1937); **60**, 989 (1938)]. We now find that this reaction may be extended to cases in which hydroxyl is replaced by $-NH_2$ or by $-NHR$, and are actively engaged in trying to determine the range of its applicability.

Specifically, serine ($HOCH_2CH(NH_2)CO_2H$) is rapidly and quantitatively split, and the dimedon derivative of H_2CO can be isolated in 95% yield. The progress of HIO_4 consumption with time is entirely consistent with the assumption that the other direct products from serine are (as would be expected) ammonia and glyoxylic acid. The latter is further oxidized, over a period of a day or two, to formic acid and carbon dioxide, according to the established reaction [Fleury and Bon-Bernatets, *J. pharm. chim.*, **23**, 85 (1936)]. Threonine reacts like serine, producing acetaldehyde, which has not as yet been quantitatively determined.

Of the naturally occurring amino acids which do not have a β-hydroxy group, tryptophan reacts rapidly with much more than one mole of periodic acid to form an insoluble product. Methionine and cystine are also somewhat rapidly attacked, but, we believe, chiefly through oxidation of their sulfur. Glycine, alanine, tyrosine, histidine, aspartic acid, asparagine, and glutamic acid reduce periodic acid at somewhat varying rates, which are estimated to be at most $^1/_{1000}$ as fast as the reaction with serine. The nature of these reactions has not yet been established, but they do not seem likely to offer any insurmountable obstacle to the use of periodic acid for the quantitative study of serine, threonine, and the somewhat hypothetical hydroxyglutamic acid in protein hydrolyzates, which we are undertaking.

As a secondary amine, diethanolamine ($NH(CH_2CH_2OH)_2$) reacts very rapidly to liberate 4 moles of formic acid. In contrast with this, diethylaminoethanol ($(C_2H_5)_2NCH_2CH_2OH$) shows practically no reaction. This behavior is probably typical of tertiary amines, and suggests that the fourth hydrogen of an ammonium ion $(R_3NH)^+$ is not sufficient to permit the desired reaction.

Preliminary results with an acylated derivative of serine indicate an extremely slow attack, the course of which is not yet definitely determined. Since, however, this last reaction could, if successful, be of even more interest in the study of protein chemistry than those already noted, our interest in it is being continued.

DIVISION OF NUTRITION AND PHYSIOLOGY
BUREAU OF DAIRY INDUSTRY BEN H. NICOLET
U. S. DEPARTMENT OF AGRICULTURE LEO A. SHINN
BELTSVILLE, MARYLAND

RECEIVED APRIL 29, 1939

PANTOTHENIC ACID AS A FACTOR IN RAT NUTRITION

Sir:

In the course of experiments designed to isolate from liver extracts a substance necessary for rat growth, it became apparent that the substance was unstable in the presence of acid and alkali, and that it could be concentrated by procedures many of which previously had been used for the isolation of pantothenic acid. Starting with 95% alcoholic liver extract, the following methods were employed: (1) extraction from acid aqueous solution by amyl alcohol and return into dilute aqueous alkali; (2) adsorption on norite and

elution with hot 60% ethanol solution; (3) continuous extraction by diethyl ether from acid aqueous solution; (4) partition of the brucine salt between chloroform and water, the activity appearing in the aqueous phase; (5) conversion of the brucine salt into the calcium salt; (6) fractionation of the latter by the procedures of Williams and co-workers [(THIS JOURNAL, **60**, 2719 (1938)].

By these means 510 mg. of white, varnish-like calcium salt (corresponding to Williams' fraction "C") was obtained from an extract derived from 160 kg. of liver. This material was fed in amounts averaging 8 mg. per week to each of six albino rats receiving a vitamin B-free diet supplemented by thiamin, flavin and the alkali-hydrolyzed eluate from fuller's earth adsorbate of liver extract. The average gain in weight for each week was as follows: (1) 13.4 g., (2) 19.1 g., (3) 18.8 g. The animals of a control group receiving the same basal diet and supplements, but without the calcium salt preparation, gained on the average as follows: (1) 6.5 g., (2) 4.5 g., (3) 4.2 g. The calcium salt preparation therefore actively stimulates rat growth.

Through the kindness of Dr. Leo Rane the calcium salt preparations were tested for their ability to stimulate the growth of *Streptococcus hemolyticus* and the diphtheria bacillus. They were found to behave like pantothenic acid preparations in stimulating the growth of both microorganisms. For these reasons it appears likely that pantothenic acid is one of the substances, in liver extracts, which are necessary for rat growth.

DEPARTMENT OF BIOLOGICAL CHEMISTRY
HARVARD MEDICAL SCHOOL Y. SUBBAROW
DEPARTMENT OF PHYSIOLOGY G. H. HITCHINGS
HARVARD SCHOOL OF PUBLIC HEALTH
BOSTON, MASS.
RECEIVED APRIL 24, 1939

PANTOTHENIC ACID AS A GROWTH FACTOR FOR THE DOCHEZ NY5 STRAIN OF HEMOLYTIC STREPTOCOCCUS

Sir:

A medium composed of gelatin hydrolyzate, amino acids, inorganic salts, glucose plus such accessory factors as glutathione, thiochrome, nicotinic acid, betaine, flavin, and glucosamine in the presence of a calcium-alcoholic precipitate of a highly purified liver extract provides almost optimum conditions for the growth of the Dochez NY5 strain of hemolytic streptococcus [L. Rane, and Y. Subbarow, *Proc. Soc. Exp. Biol. Med.*, **38**, 837–839 (1938)]. We have found that the further addition of uracil, guanylic acid, xanthine, hypoxanthine, nicotinic acid amide in place of nicotinic acid, and a fraction of liver extract as yet unidentified may also be of significance in the growth of this strain of hemolytic streptococcus.

Certain similarities in the isolation and properties of the unknown factor in the liver extract and pantothenic acid suggested the possibility of substitution. Pantothenic acid "U-6000, *ca.* 50%," kindly supplied by Dr. R. J. Williams, has been tried. Pantothenic acid is active in the growth of the Dochez NY5 strain of hemolytic streptococcus, as indicated in the table. The amount of growth was equal to that obtained with the calcium-alcoholic precipitate of liver extract as described in our previous publication.

Pantothenic acid per 10 cc. basal medium, γ	100	50	25	10	5	2.5	1	0.5
Nephelometer reading (cf. L. Rane, and Y. Subbarow, —*loc. cit.*)	2.9	2.9	2.8	2.8	2.8	2.9	3.5	>4.7
Control, growth of organism in meat infusion broth	2.3							

It is of additional interest that a product synthesized in collaboration with G. H. Hitchings of the Harvard School of Public Health is able to replace pantothenic acid in an otherwise chemically-defined medium. The compound was made by the conjugation of β-alanine ethyl ester with the acyl chloride of acetylated α,δ-dihydroxyvaleric acid. The dihydroxyvaleric acid was obtained by the deaminization of *d*-ornithine. However, the material so prepared was needed in larger amounts than was pantothenic acid.

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JAMAICA PLAIN, MASS.
RECEIVED APRIL 24, 1939

REACTION OF NEOPENTYL CHLORIDE WITH SODIUM

Sir:

We have isolated from the reaction of one mole of neopentyl chloride and sodium, a 13% yield of 2,2,5,5-tetramethylhexane, b. p. 135° at 736 mm., n^{20}D 1.4049, a 36% yield of neopentane, f. p. –19 to –20°, b. p. 8.3° at 720 mm., and 17.6 g. of a

substance, b. p. 19.8° at 740 mm., $n^{20}D$ 1.3656, $d^{14.4}_4$ 0.6681. This substance dissolved in 66% sulfuric acid at 0° within ten minutes and did not decolorize a dilute alkaline permanganate solution within twenty-four hours. These properties correspond with those reported for 1,1-dimethylcyclopropane (Gustavson and Popper, *J. prakt. Chem.*, (2) **58,** 458 (1898)); the yield corresponds to 25%

Because of similarity in physical constants, the possibility of this compound being isopropylethylene was recognized. A known sample of isopropylethylene, prepared from isoamyl chloride and alcoholic potassium hydroxide, had the properties b. p. 18.8° at 731 mm., $n^{20}D$ 1.3640, d^{15}_4 0.6332. It was insoluble in 66% sulfuric acid and decolorized a dilute alkaline permanganate solution instantly under the same conditions as used for the 1,1-dimethylcyclopropane above.

The significance of these results for the current theory of intramolecular rearrangement, especially in relation to the formation and behavior of free radicals, will be discussed in a separate paper.

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RECEIVED APRIL 21, 1939

NEW BOOKS

Vitamin B₁ (Thiamine) and its Use in Medicine. By ROBERT R. WILLIAMS, Sc.D., Bell Telephone Laboratories, and TOM D. SPIES, M.D., Associate Professor of Medicine, University of Cincinnati. The Macmillan Company, 60 Fifth Avenue, New York, N. Y., 1938. xvi + 411 pp. 19 figs. 16 × 24.5 cm. Price, $5.00.

A very excellent Macmillan Medical Monograph presenting a history of the discovery of vitamin B_1 (Thiamine), its function in the regulation of cell respiration, and other physiological data, which are of immediate interest to clinicians and practicing physicians. It is an inspiring story of medical and biochemical progress beginning with the Chinese description of the disease beriberi—in the early centuries, and revealing the successive contributions of workers during years of scientific research finally leading up to the determination of structure and complete synthesis of the neutral vitamin substance by American investigators.

The authors have summarized the present knowledge of this important chemical substance in convenient form for future reference. In Part I are recorded data that are of the greatest interest and value to all practitioners of medicine. Particular attention is paid by the authors to the clinical considerations of practical interest and importance; to the relation of beriberi to similar diseases, the pathology and physiology of vitamin B_1, its pharmacology, the methods of prevention and treatment of vitamin B_1 deficiencies and the relation of diet to beriberi disturbances.

In Part II the authors have presented a very complete survey of the chemical and biological literature dealing with vitamin B_1. This embraces a review of its discovery, the methods of isolation and identification from natural sources, the determination of constitution and its final synthesis. The authors review also in this part the nature of the functional groups of the vitamin B_1 molecule; the adopted methods of biochemical analysis, and discuss the present conclusions of physiologists regarding thiamine requirements in nutrition, and its general distribution in the living organism. Thiamine is one of several organic substances which play a vital role in living cells. The authors have brought together a large amount of scientific data of immediate interest to chemists, physicians, clinicians, physiologists and biologists. They deserve much credit for their method of presentation and its completeness. A most commendable feature of the book are the excellent bibliographies introduced at the end of each chapter.

TREAT B. JOHNSON

Feuerfeste Baustoffe silikatischer und silikathaltiger Massen. (Refractory Materials of Construction Made up of or Containing Silicates.) By Dr.-Ing. Dr. Phil. CLAUS KOEPPEL. Verlag von S. Hirzel, Königstrasse 2, Leipzig C 1, Germany, 1938. xvi + 296 pp. 51 figs. 15 × 23 cm. Price, RM. 15.50; bound, RM. 17.

This is Band 18 of "Chemie und Technik der Gegenwart" ("Modern Chemistry and Technology"). The author's purpose, freely translated, is "to help both the student and the practical man to orient himself in the wide-extending field of the silicate sciences with particular reference to their application to current practice in the manufacture and handling of refractory materials of construction." The book will be much easier reading for the experimental chemist than for either the student or the practicing ceramist, for the language is that of the scientist rather than the technician or the engineer. Nevertheless, the plan of treatment indicated above is carried out quite thoroughly.

The principal emphasis is on the properties and reactions of pure silica. This body of knowledge not only is